chapter 2

GENES AS DETERMINANTS OF HEREDITY

Darwin thought of evolution as a process of adaptation to environment by means of the natural selection of favorable "variations." Within the context of the knowledge of his day he could not, of course, replace the word "variations" with "mutations," since the science of genetics had not yet been invented. However, being a man with a strong urge to tie up loose ends, Darwin suggested that "variations," including those that he felt might be acquired in response to environmental pressures during the lifetime of the organism, were inherited by a mechanism in which all the somatic (body) cells contributed information to the germ cells. We know now that acquired characteristics are *not* inherited and, with the emergence of genetics, it became possible to speak of the inherited characteristics of an organism (his phenotype) as the expression of the sum of his chromosomal genes (his genotype).* We may now

* It should be stressed that environmental conditions, during development, can exert a profound influence on the phenotypic expression of the genes. A classical example of this is the effect of temperature on the number of eye facets in *Drosophila* whose chromosomes bear the mutations "low-bar" and "ultra-bar."[1] Two organisms with identical developmental potentialities may *look* or *act* quite differently, although their respective offspring will be back to the old standard

describe evolution in terms of the natural selection of favorable gene mutations in a population and the perpetuation of these through reproduction.

Since this book is directed at biochemists, many of whom may have had as little formal training in genetics as I have, it is necessary to present, as a starting point for further reading, an abbreviated survey of the gene concept and of some of its experimental consequences. We shall restrict ourselves to the Mendelian genetics of normal bisexual reproduction as it occurs in the higher plants and animals. The mechanisms involved, although by no means universal, can serve as a qualitative basis for considering the reproduction of even such specialized genetic systems as the bacterial viruses, if we are willing to cut some corners.

Nearly a hundred years ago, Gregor Mendel made the observations that established the fundamental laws of genetics. Mendel crossed strains of garden peas which differed in one contrasting character (e.g., purple or white flowers) and observed that the progeny (the so-called F_1 generation) were all purple. This character was, then, the "dominant" trait and white the "recessive." Similar dominance or recessiveness was observed for many other alternative traits.

When two members of the F_1 generation were crossed, he observed that about three-fourths of the progeny in the F_2 generation were purple and one-fourth white. These experiments suggested that any particular character-determining unit of heredity exists in two forms and that these *"allelic"* forms do not blend but maintain their identity throughout the life of the F_1 organisms to separate later in the following generation. The units of heredity were subsequently named "genes" by Johanssen in 1911. An organism, like the F_1 peas of Mendel, which contains both allelic forms is said to be a *heterozygote,* and those possessing a double dose of one or the other allele is a *homozygote.* We refer, genetically, to the former as *Rr* and to the latter as *RR* or *rr* (homozygous for the dominant and recessive forms respectively).

Mendel's experiment, summarized in Figure 9, illustrates the "law of segregation." The frequency of occurrence of purple and white flowered plants in the F_2 generation (3:1) is to be expected if the two allelic forms of this particular color-determining gene, one dominant over the other, segregate to yield equal numbers of *R* and *r* units during the formation of germ cells and then proceed to recombine at random in the new generation. Mendel checked this hy-

and the superficial characteristics acquired as the result of environmental pressures will not be inherited.

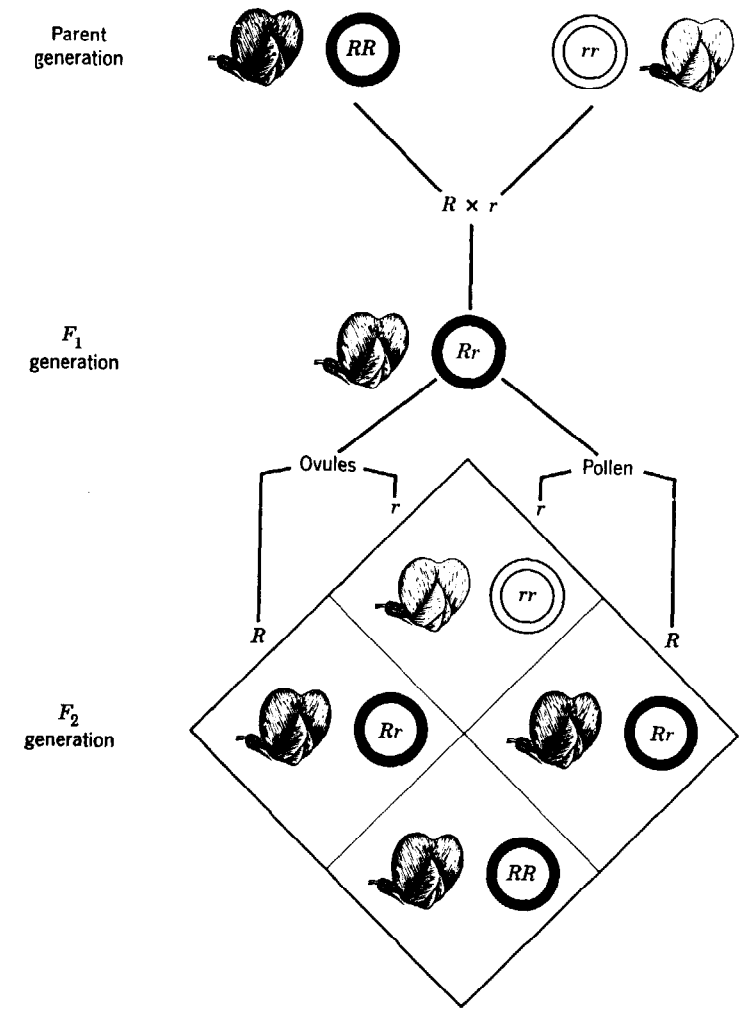

Figure 9. Mendel's first law, the law of segregation; *R* stands for the gene for purple and *r* for the gene for white flower color. Black rings and white rings symbolize purple and white-flowered plants, respectively. Purple color is dominant over white. Redrawn from T. Dobzhansky, *Evolution, Genetics, and Man,* John Wiley & Sons, 1955.

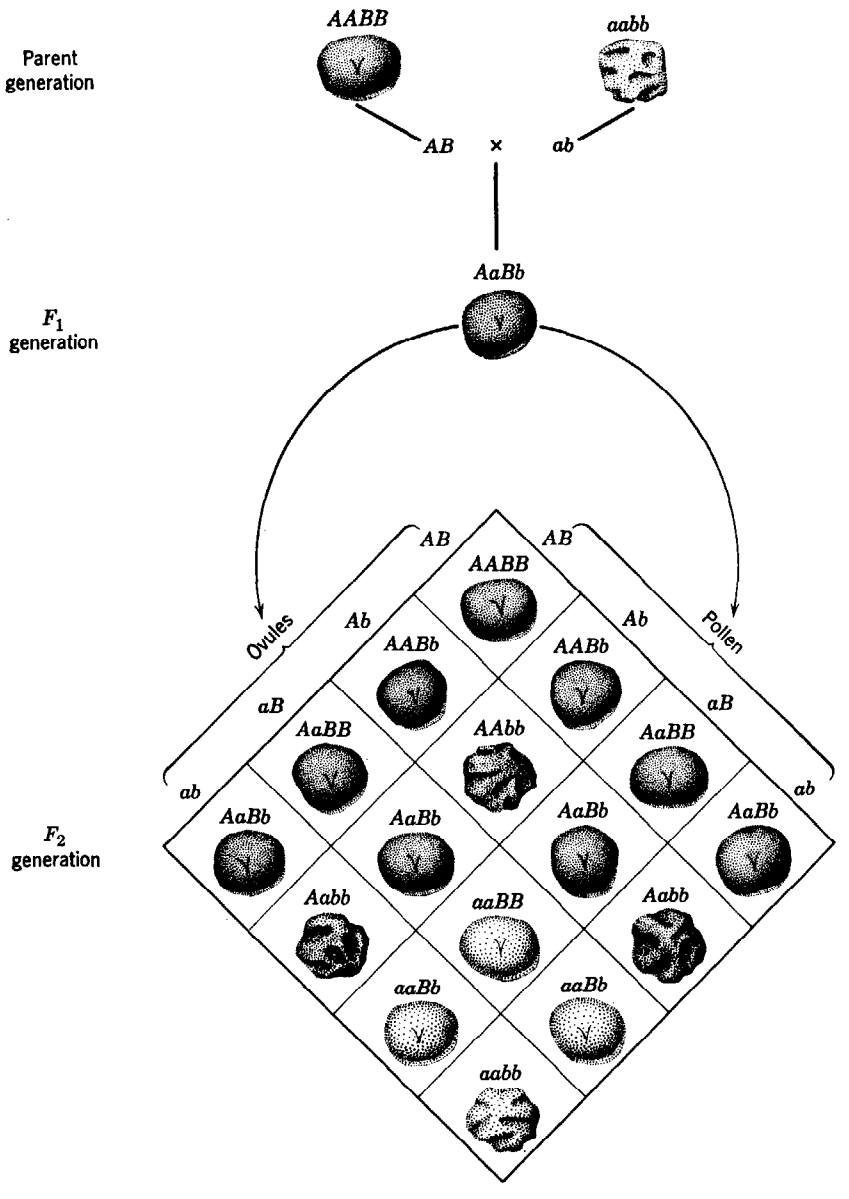

Figure 10. Mendel's second law, the law of independent assortment; *A* and *a* represent the genes for yellow and green colors, respectively, and *B* and *b* those for smooth and wrinkled seed surfaces. Yellow is dominant over green and round is dominant over wrinkled. Redrawn from T. Dobzhansky, *Evolution, Genetics, and Man*, John Wiley & Sons, 1955.

pothesis by allowing the *purple*-flowered plants in the F_2 generation to produce an F_3 generation. One-third of the F_2 plants (the *RR* strain) produced only purple-flowered progeny, whereas two-thirds (the *Rr* variety) produced either white- or purple-flowered progeny in the ratio 1:3 as predicted by the principle of segregation.

In some of his experiments Mendel crossed peas which differed in two or more traits. Thus, as summarized in Figure 10, he crossed peas having yellow, smooth seeds with others having green, wrinkled seeds; he knew in advance that the gene for yellow color was dominant over that for green and the gene for round seeds was dominant over that for wrinkled. The F_1 generation had seeds which were yellow and smooth, since both dominant traits were present in this hybrid and determined the phenotype. In the F_2 generation, however, the phenotype was determined by a random combination of the four segregated traits as shown in the figure. Seeds of the F_2 progeny showed all the four possible combinations of phenotype but, because of the dominance of yellow and smooth over green and wrinkled, these appeared in a ratio of 9:3:3:1 with only one-sixteenth of the seeds having the double recessive characteristics. This phenomenon, *independent assortment* of genetic traits, is the second basic "law" growing out of Mendel's studies.

The simplicity of Mendel's experiments and their ease of interpretation were really due to his good fortune in choosing sets of traits which segregated and recombined to give the theoretical 3:1 ratio. In many instances this ratio is not obtained, and instead certain sets of genes may segregate together to yield what are termed "linked" traits. To understand the linkage of genes we must first consider the phenomena of *mitosis* and *meiosis*.

Cytologists have been aware for over a hundred years of chromosomes as visible rod or thread-like structures that appear in the nucleus during cell division. The number of chromosomes per nucleus is a characteristic constant for any given species. The genetic information present in a cell is accurately perpetuated in each of the daughter cells by the process of *mitosis*. The stages in mitosis are shown in Figure 11 as they are observed in the root tips of the common onion. The simplified drawing on the left side of the figure depicts the behavior of a single chromosome of this plant. The centromeres are represented, in this figure, by open circles. These specialized structures within each chromosomal strand act as points of attachment for the fibers which bind the chromosomes to the pole of the spindle during subdivision of the cell. The centromere is replicated during the division cycle, as shown. Occasional

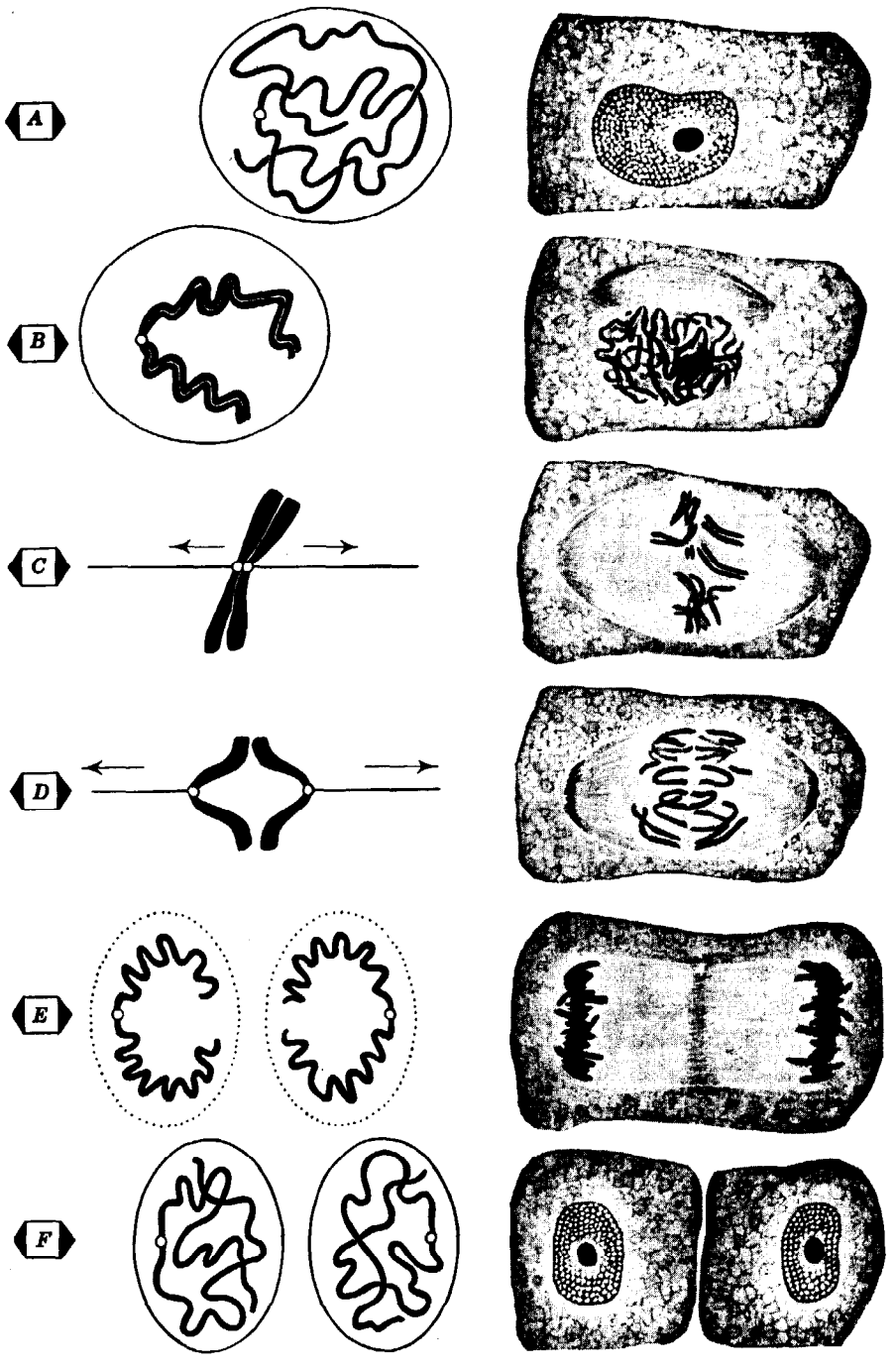

Figure 11. Mitotic cell division in the common onion: *A*, interphase; *B*, prophase; *C*, metaphase; *D*, anaphase; *E*, telophase; *F*, daughter cells. From T. Dobzhansky, *Evolution, Genetics, and Man*, John Wiley & Sons, 1955.

cells containing chromosomes which lack a centromere, or which have more than one, do not survive. The genetically significant event is the exact duplication of each chromosomal daughter-strand during the period between stages *F* and *B*, whereby hereditary constancy is insured in all the somatic cells of an organism during its growth and development.

The nucleus of the somatic cell (diploid) contains twice as many chromosomal strands as the germ cells or *gametes* (haploid). The complement of chromosomal strands in a gamete is the same as that of somatic cells immediately following mitosis, before the machinery of the cell has had an opportunity to bring about duplication of each strand. That is, each gamete contains only a single allelic form of each gene. When two sex cells unite, the resulting diploid *zygote* contains the hereditary units of both parents arranged in such a way that the corresponding chromosomal strands are paired with each set of allelic genes in exact physical complementarity.

When the time comes for the cells of the reproductive tract to produce gametes, there occurs a process termed *meiosis*, which is summarized schematically in Figure 12. The sets of chromosomes first enter a stage resembling prophase in mitosis. The corresponding maternal and paternal chromosome sets then proceed to find one another by a miraculous procedure in which each bit of cytologically discernible detail along the maternal strand pairs with its opposite number in the paternal strand. Each of the two strands then subdivides into two, and, in most organisms, the pairs of strands are bound together at one or more points by "chiasmata" (Figure 12*D*).

The further stages of meiosis lead to the formation of gametes containing only one chromosome of each kind. As shown schematically in the figure, the centromere divides during the second meiotic division. The details of these latter stages of meiosis are somewhat different in different organisms, but the end result, haploid sex cells, is the same.

Early in this century cytologists recognized that the phenomena of independent assortment and segregation of heritable characteristics were consistent with the behavior of chromosomes during cell division. Direct evidence for such a correlation was soon forthcoming, largely through the efforts and imagination of T. H. Morgan. Morgan chose as his experimental object the fruit fly, *Drosophila melanogaster*, which contains extremely large chromosomes in the cells of its salivary glands. This organism possessed a number of important advantages for genetic research, including a high rate of multiplication and a genetic apparatus having only four pairs of chromosomes.

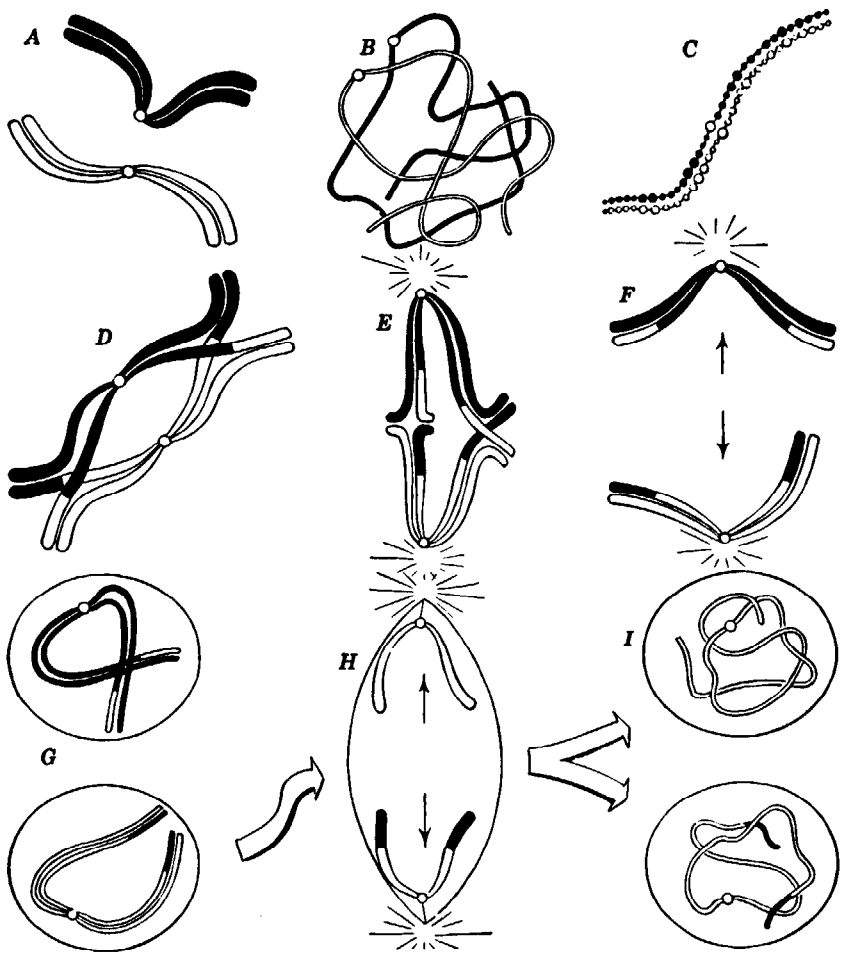

Figure 12. Schematic design of the stages of meiosis. Only a single pair of chromosomes is shown. The paternal chromosomes are in black and the maternal in white. The centromeres are shown as white circles. After T. Dobzhansky, *Evolution, Genetics, and Man*, John Wiley & Sons, 1955.

By crossing strains of flies which showed different inherited traits, Morgan demonstrated that many of these traits behaved according to the principles of Mendelian genetics. He soon observed, however, that a number of traits did *not* show independent assortment but were frequently transmitted from parent to progeny as though they were *linked* together in a genetic bundle. A consideration of the scheme in Figure 12 will make clear the (correct) explanation put forward

by Morgan for these observations. Except for the segments of each chromosomal strand that may be exchanged for their counterparts in the course of the formation of chiasmata, the total genetic information in each chromosome appears in any specific gamete as a unit. Thus, two closely linked genes (and we may think of this linkage, in physical terms, as distance along the strand) are not likely to become separated from one another during meiosis. Morgan and his scientific followers in the field soon found that the traits with which they dealt fell into four linkage groups and concluded that each corresponded to one of the four chromosomes. This conclusion was completely supported when subsequent studies on the giant salivary gland chromosomes of *Drosophila* made possible the direct comparison of gene mutations as detected by genetic analysis with visible morphological changes in the individual chromosomes themselves (Figure 13).

Genes that are linked together frequently do show independent assortment, in spite of their location on the same chromosome. This separation is explainable in terms of the exchange of chromosomal segments that takes place between the two strands during the formation of chiasma. (See transfers indicated in Figure 12*D*.) Morgan suggested that the frequency of separation, or of recombination, of two linked genes is a function of the linear distance separating them. Stated in other terms, the probability of a chiasma occurring between two distant genes would be much greater than the probability of one occurring between two genes which are close to one another. His hypothesis has been amply confirmed by a vast amount of data on the recombination of linked genes in a variety of organisms and, although there exist numerous examples of quantitative deviation from the rule, frequency of recombination is in general a reliable measure of the separation between genes.

At this juncture it may be wise to introduce an aside directed toward the novice in genetics. The picture we have drawn of the development of the fundamental concepts of genetics has been made purposely rosy for simplicity's sake. In this discussion, and in what follows, we are interested in getting across only the most basic conceptual framework of the subject and cannot consider the many reservations and qualifications to be found in any adequate textbook. (For example, in male *Drosophila* no chiasmata are formed during the process of spermatogenesis, and consequently no linked genes can undergo recombination in the progenies of hybrid males. In the reproduction of bacteriophage, a matter we shall discuss at greater length in later chapters, recombination of linked genes

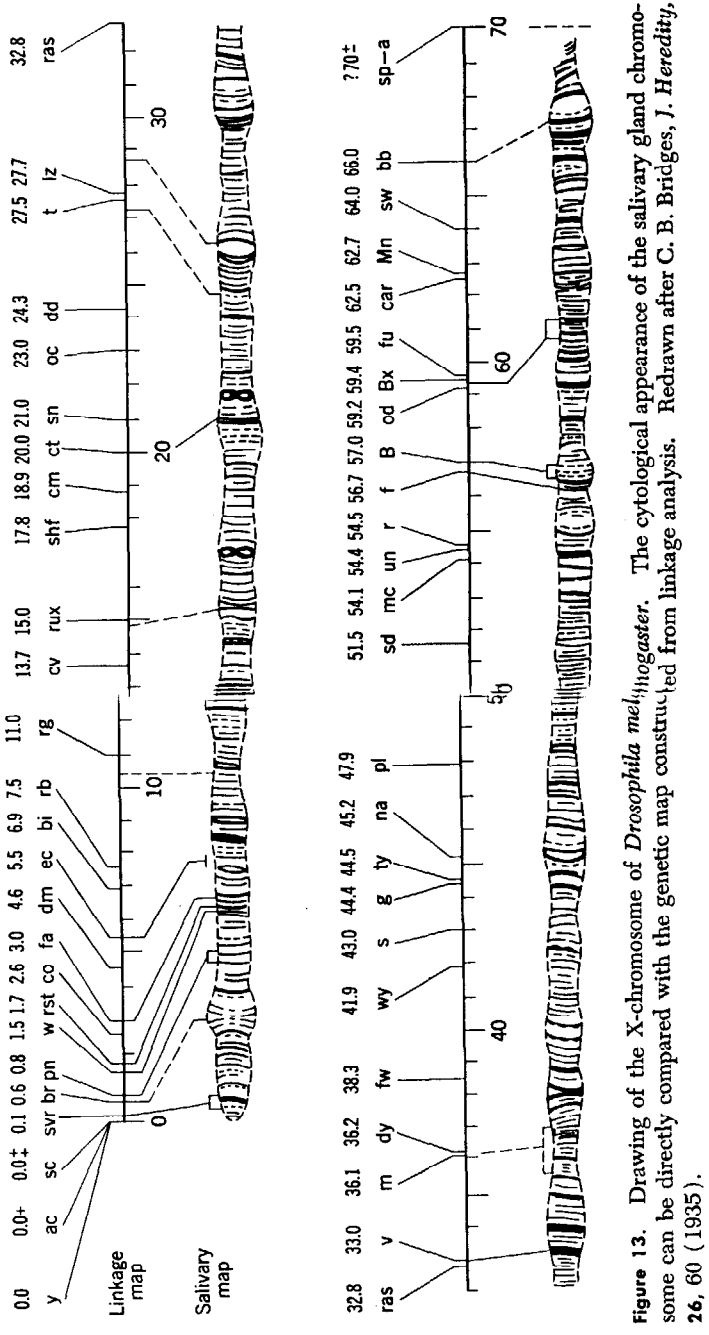

Figure 13. Drawing of the X-chromosome of *Drosophila melanogaster*. The cytological appearance of the salivary gland chromosome can be directly compared with the genetic map constructed from linkage analysis. Redrawn after C. B. Bridges, *J. Heredity*, **26**, 60 (1935).

takes place, *from a statistical point of view*, in a manner quite analogous to recombination in higher organisms. Estimates of the distances between two genes on the phage "chromosome" may be based on the same general sort of calculation that we employ for studies on sweet peas, in spite of the fact that classical reciprocal crossover does not occur; that is, wild-type and double recombinant phages do not, both, generally result from a single mating event.)

If genes may be thought of as being arranged in a linear fashion along the chromosomal strand, and if the distances between them may be estimated by linkage analysis, it is clear that a "map" can be constructed expressing their physical relation to one another. Such maps have been prepared for a number of species of higher organisms and more recently for bacteria and viruses as well. A map of some of the genes that have been studied in *Drosophila melanogaster* is shown in Figure 14. In general, the distances indicated between genes can be shown to be qualitatively correct by internal checks. Thus, in a series of crosses involving three genes *A*, *B*, and *C*, if it is found that the distance between *A* and *B* is x units and between *B* and *C* is y units, the distance between *A* and *C* will be found to be approximately x plus y units. The units used here are "units of recombination" and are merely the percentage of the progeny from any particular cross that is different from either parent genotype. For a variety of reasons, the "genetic distances" indicated on maps such as that shown in Figure 13 bear only a rough correspondence to the actual physical parameters of the chromosomal strand. One factor responsible for such deviations is the apparent greater potentiality of some parts of the chromosome to crossover than others. Another factor involves the occurrence of multiple crossovers. As the length between two genes becomes larger and larger, the chance of multiple crossovers will increase and, in the limit, there will be an equal chance of an even number and an odd number of crossovers. Thus with widely separated genes and with random crossover, the "map distance" would approach 50 recombination units rather than 100. Genetic maps appear, in general, to be a reliable representation of the relative order of genes, confirming the concept of a linear arrangement. But it must be recognized that the frequency of crossover varies from point to point along the chromosome, and from species to species, and has great influence on the additivity of distances and on the total apparent map length.

In the vast majority of cases, the translation of phenotype into the language of genetics follows the simple rules we have attempted to summarize. The difficulties experienced by nonspecialists in the

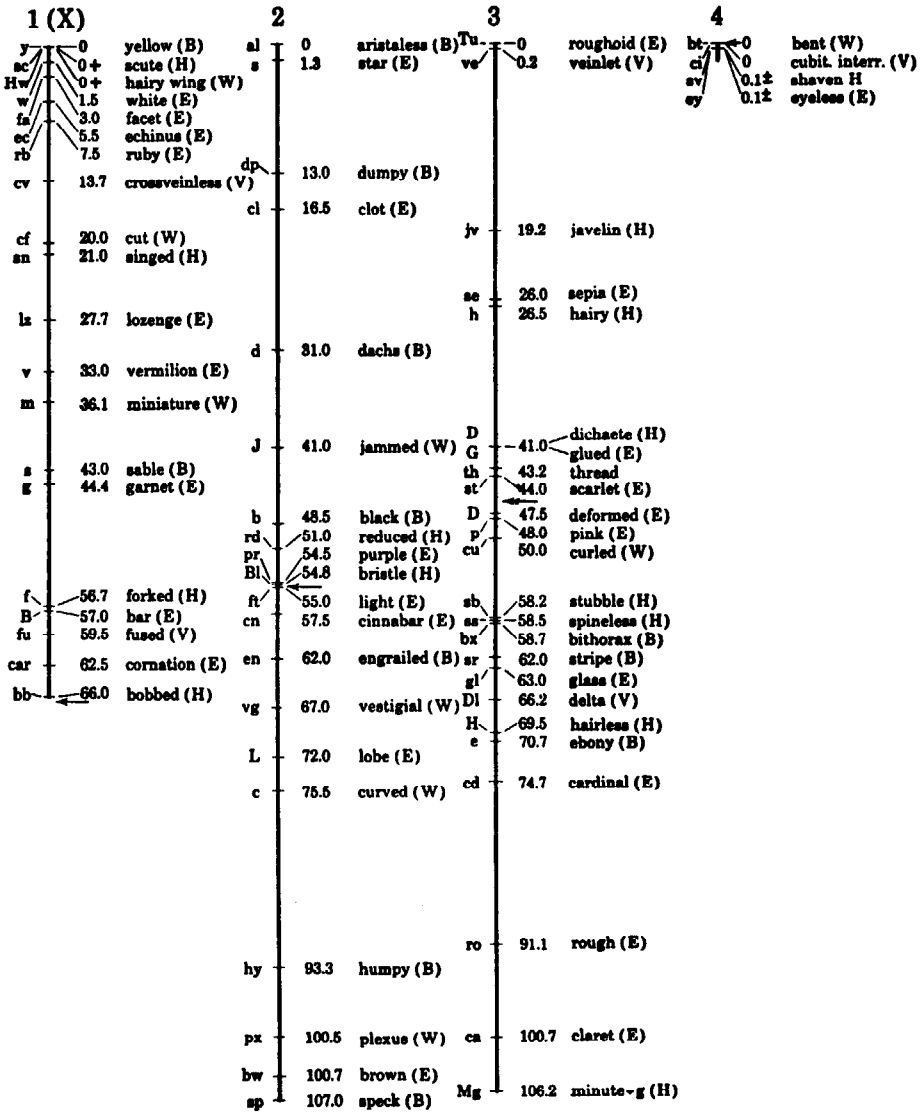

Figure 14. Genetic maps of the chromosomes of *Drosophila melanogaster*. After C. Bridges, from Mary J. Guthrie and John M. Anderson, *General Zoology*, John Wiley & Sons, 1957.

course of reading genetic literature arise from the terminology which has been needed by experts to categorize the abnormal. A gene is recognized only because it can be modified and appear in an abnormal allelic form which determines some unusual phenotypic character. We refer to such changes in genes as *mutations*, but we must be constantly aware of the fact that the word has a multiplicity of meanings and that true understanding of genic modification can only be reached when the genetics becomes describable in chemical terms. The appearance of a new phenotypic character may be due to a change in the gene itself, chemical or configurational, to a deletion or reduplication of the gene, or to one of a number of "position effects" involving the inversion or translocation of genes to new positions along the chromosome. As stated by T. Dobzhansky,[2] "A chromosome is not just a container for genes but a harmonious system of interacting genes. The arrangement of genes in a chromosome has developed gradually during the evolution of the organism to which the chromosome belongs; the structure of a chromosome, like the structure of any organ, is a product of adaptive evolution." It is to be hoped that the foregoing discussion of the simplest elements of genetics will be sufficiently irritating in its compactness (and incompleteness) to cause some readers of this book to look into a few of the volumes listed at the end of this chapter.

Most of what follows in this book will be concerned with what genes do, and we approach the subject in terms as chemical as possible within the limits of our present knowledge of nucleic acid and protein structure. In the classical sense, the term "gene" has a purely operational meaning. It may be applied to any unit of heredity that can undergo a mutation and be detected by a change in phenotype. As the determined distances between genes on chromosome maps become less and less, the maximum size of the chemical unit which determines a gene must be thought of as being smaller and smaller. Our impression of the size of a gene, from genetic information alone, depends entirely on the sensitivity of the methods available for the detection of extremely infrequent crossovers. It is precisely within this twilight zone of detectability that the classical definition of the gene begins to break down; here contemporary research in genetics and chemistry finds common ground. Estimates of the size of a gene (as an operational unit) have been made by several methods which together more or less define the upper and lower limits. One sort of estimate is possible from crossover data. Muller and Prokofyeva,[3] for example, localized four genes on the giant salivary gland chromosomes of *Drosophila* within a distance of 0.5 micron and concluded

that the upper mean limit of length for each must therefore be 1250 A. Other estimates, derived from studies of the effects of ionizing radiation on the frequency of mutation, indicate that a single gene may occupy a volume corresponding to a sphere with a diameter as small as 10 to 100 A. The discrepancy between crossover and radiation data is considered too large to be due to experimental or interpretative error and suggests that two different aspects of gene structure are being measured by the two techniques, one having to do with the crossover of the entire, intact gene (that is, a functional unit of genetics) and one with the modification of chemical fine structure within its macromolecular architecture.

This conclusion appears to be supported by recent developments in genetic fine-structure analysis, a few of which we shall review subsequently. To establish a bridge between the more classical concepts of genetics and the rather revolutionary findings of the contemporary microbial geneticist, it is instructive to consider an example of the apparent subdivision of a single gene in the genetic material of *Drosophila.*

In the course of linkage analysis, certain genetic units called "pseudoalleles" have been detected which appear to be concerned with the same, or at least with a closely related, function. One such set of pseudoalleles makes up the "lozenge genes" of *Drosophila melanogaster.* A mutation in the "lozenge" region causes changes in the pigmentation of the eyes and also certain other morphological changes. The mutant forms are recessive to the normal allelic form of the gene; that is, heterozygotes show normal pigmentation. Green and Green[4] have studied three mutational loci within this region of the genetic map, all of which have "lozenge" characteristics. From an analysis of crossover data, they have determined that all three loci fall within a genetic distance of less than 0.1 units of recombination. They were further able to show that *double* heterozygotes, in which the *two* mutant alleles were on the same chromosomal strand, showed the wild-type character, whereas an arrangement in which the two mutants appeared on different strands of the same chromosome produced the mutant phenotype. The phenotypic consequences of the various arrangements of two mutant loci are shown in Figure 15. Two explanations for these observations have been offered. One suggests that each of the individual loci controls a different enzymatic activity which is in close physical association with the genetic locus itself. These enzymes are pictured as components of a series of consecutive reactions leading to the formation of an essential chemical material. Such a situation might apply if the individual re-

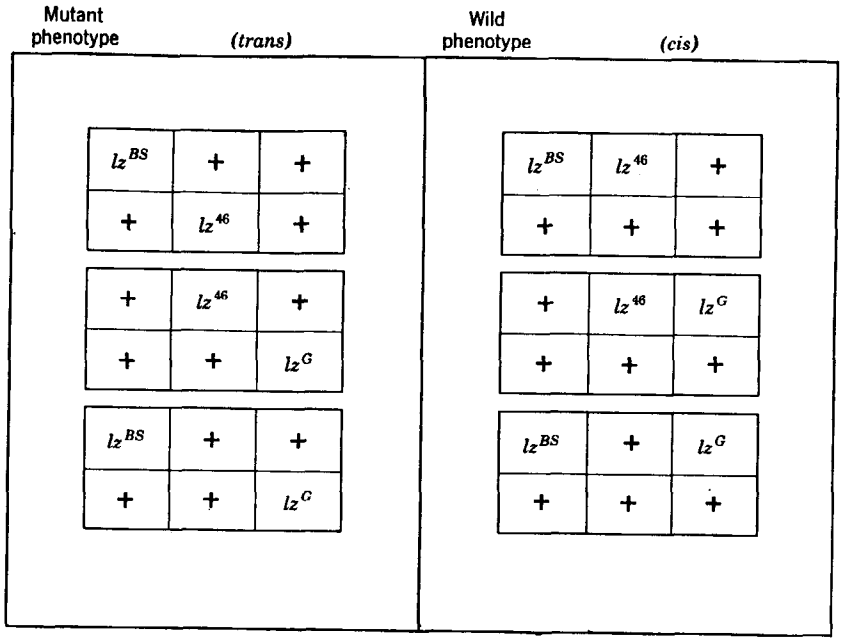

Figure 15. Schematic diagram of the *cis* and *trans* arrangements of the pseudoallelic "lozenge" genes in *Drosophila melanogaster.* After M. M. Green and K. C. Green, *Proc. Natl. Acad. Sci. U.S.,* **35,** 586 (1949).

actions were of such a nature that the operation of the reaction chain depended on certain minimum concentrations of intermediates and would be interrupted should diffusion (from one chromosomal strand in the "mutant" heterozygotes of Figure 15 to another, for example) lead to a suboptimal concentration level for any of the intermediates. This explanation for pseudoallelism clearly involves a number of rather large assumptions and seems less likely, at the moment, than the second alternative, namely, that each pseudoallelic mutation, although distinguishable, like any "gene," by crossover, is really a change in the *substructure* of the functional parent gene. Thus, we may postulate that a mutation at any of the three loci of the lozenge gene might equally impair its function and that only with the *cis* arrangement, in which one complete unmarred strand carries the load, can the normal phenotype be expressed. To anticipate some of our later discussion, this idea has been used by Benzer as the basis for the coining of a new genetic term, the "cistron," by which is meant a genetic *unit of function* subdivisible by genetic

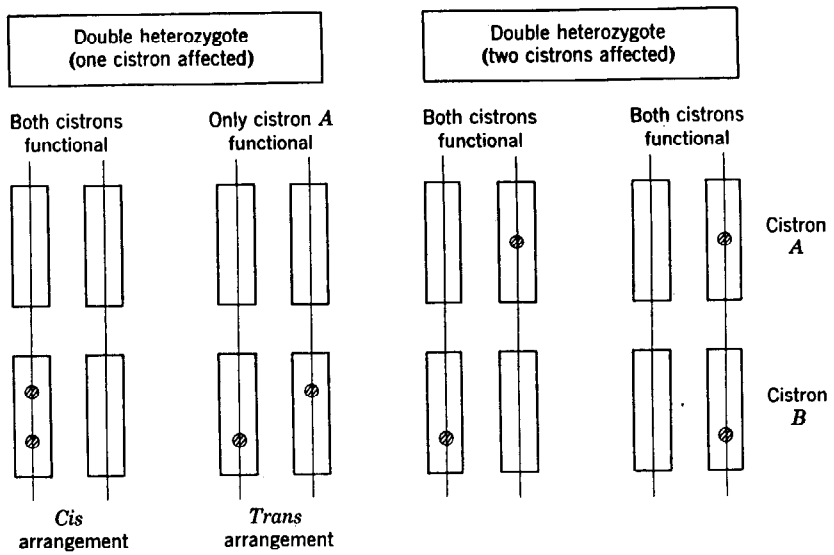

Figure 16. The subdivision of a functional gene into "cistrons," both of which must cooperate to produce an expression in the phenotype. When two mutations occur in the *same* cistron, normal function can only be expressed when the loci are in the *cis* arrangement, but not when they are in the *trans* arrangement. Based on the suggestions of Seymour Benzer, *The Chemical Basis of Heredity,* Johns Hopkins Press, 1957.

tests into ultimate units of recombination termed the "recon." In this system of terminology, two recons would belong to the same cistron when the *cis* arrangement of two mutant loci in a double heterozygote (Figure 16) results in functional adequacy and the *trans* arrangement does not. The demonstration that genes are made up of blocks of very closely linked subunits which may be differentiated by crossover has been a tremendous stimulus to biochemists interested in "genetic chemistry." The mutational effect of ionizing radiation on a bundle of genetic matter having an estimated diameter of 10 A. or so becomes a much more tangible phenomenon when we can compare such a distance with equivalent chemical distances, such as the separation of side chains on a polypeptide or the molecular dimensions of a dinucleotide. As we shall discuss in later chapters, the ultimate mutable units of genetics do, indeed, appear to be about this size, and it is possible that we may soon be able to equate them with individual nucleotide residues along the polynucleotide strands of deoxyribonucleic acid.

No summary of genetic principles would be complete without some discussion of heredity in *Neurospora. Neurospora* occupies a special niche in genetics because a great deal of the evidence relating genetic constitution to biochemical behavior has been obtained through its study.

It has long been evident that mutations are reflected as changes in biochemical properties. This is essentially a paraphrase of the statement that mutations are detected only because of the difference in phenotype which they induce, the phenotype of an organism presumably being the sum of its biochemical potentialities. The studies on the genetic control of the structure of flower pigments by Lawrence,[5] Scott-Moncrieff, and their colleagues helped establish the fact that individual genes determine the exact chemical structure of these pigments by regulating the extent of methoxylation, hydroxylation, or conjugation with carbohydrate of certain heterocyclic compounds called anthocyans. These studies already began to suggest that the modification of a single gene leads to a change in some specific biosynthetic process.

Wild strains of *Neurospora* may be selected which will grow well on an extremely simple culture medium consisting essentially of sugar, salts, and a single vitamin, biotin. By exposing such cultures to some mutagenic agent (e.g. X-rays), we obtain mutants that no longer grow on the minimal medium but require the addition of nutritional additives like yeast extract and hydrolyzed proteins and nucleic acids. By systematic dissection of the additive mixture, it may be determined which single nutritional requirement has been induced by mutation. The isolation of mutant forms having clear-cut nutritional requirements is not always simple, and many have been isolated which undergo spontaneous reversion to the wild type or which continue to grow on a minimal medium, although at a much reduced rate. However, a large number of stable, full-blown mutants that require a single nutritional additive for growth has now been isolated. These nutritional substances include a variety of amino acids, purines and pyrimidines, and vitamins. Because of the conventional chromosomal system of inheritance, the position of these mutant loci in *Neurospora* may be established by orthodox crossing over methods. The experimental approach to mapping is indicated by a consideration of the natural history of *Neurospora,* the main points of which are shown in Figure 17.

In *asexual* reproduction, the haploid conidia germinate to produce

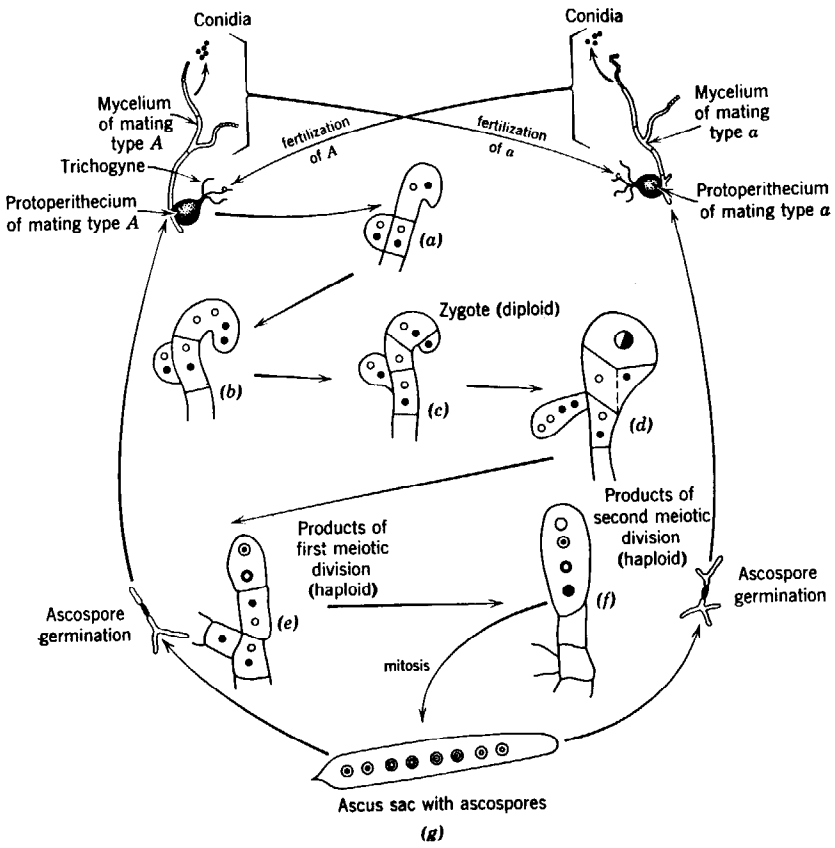

Figure 17. The life cycle of *Neurospora crassa.* The genetic events which occur during the first and second meiotic divisions are illustrated in greater detail in Figure 18. Redrawn, in part, from R. P. Wagner and H. K. Mitchell, *Genetics and Metabolism,* John Wiley & Sons, 1955.

more haploid mycelia. Increase in mass also takes place by simple growth of existing mycelia through mitosis and the utilization of nutrients from the culture medium. In sexual reproduction cross-fertilization takes place between two mating types, variously referred to as *A* and *a*, or + and −. The conidia of these two types appear to differ only in a single genetic locus on one of the chromosomes. In a cross, the haploid nuclei of the two mating types become associated within a common cytoplasm. In subsequent events (Figure 17) the nuclei of both mating types undergo numerous equational divisions (*a*,*b*) and subsequently fuse, side by side, into a diploid pair

(*c*,*d*). This zygote (*d*) then undergoes two rounds of meiosis (*e*,*f*) to produce four haploid nuclei (*f*) which then divide mitotically, to yield eight ascospores (*g*). When these ascospores are exposed to heat or to certain other stimuli (furfural), germination is induced.

One of the advantages of *Neurospora* as an experimental tool in genetics is the fact that the order of events during meiosis is faithfully mirrored in the final asci. As summarized in Figure 17, the upper and lower sets of two nuclei at the four-nucleate stage are derived from the upper and lower nuclei of the binucleate state, and a similar regularity is preserved after the subsequent mitotic division (stage *g*). The individual ascospores may be dissected out by hand, in order.

With some mutations, which cause a visible difference in the appearance of the final ascospore, we may estimate, without testing the individual spores, the frequency of crossing over during meiosis, and thus the map position of the locus in question in relation to the centromere as a zero point. This procedure is illustrated in an elegant way by an example taken from the work of D. R. Stadler[6] on an unusual lysine-requiring mutant. This mutant, one of a number of lysine-requiring strains studied by N. Good in 1951, exhibits delayed ascospore formation, and mutant spores may be detected within the ascus by their colorless appearance. Perpetuation of this abnormal strain is possible, in spite of the arrested maturation, because the vegetative mycelium can be cultivated indefinitely without the necessity for sexual reproduction and also because an occasional mutant spore will mature upon aging. The photograph in Figure 18 shows the typical appearance of the asci that are produced when the mutant is crossed with a wild-type strain.

The critical stages in meiosis following the cross are shown schematically in Figure 19. The two haploid conidia first fuse to form a zygote a_1. (This zygote is known to be in a double-stranded form (a_2) at the start of the first meiotic division.) During this first meiotic division crossover may or may not occur between the two sets of parental strands. In *Neurospora,* the centromeres from each parental chromosome do not divide during the first meiotic step, and the crossed-over pairs of strands remain attached as shown in the figure (*b* and *c*). The frequency of crossing over of a given allele during the first meiosis is assumed to be a function of the distance of this locus from the centromere.

During the second meiotic division each nucleus yields two daughter nuclei to give a total of four, arranged in a row, the upper and lower set derived by division of the upper and lower of the two

nuclei in the binucleate cell. If no crossover has occurred, the order shown in *d* develops, whereas *with* crossover four different arrangements may be obtained (*e*). When the four-nucleate cells undergo subsequent mitosis, various asci are produced as shown in the photograph.

The spores containing the mutant locus are easily distinguished by their colorless appearance. Inspection of the photograph (Figure 18) indicates that in nine of the fourteen mature ascospores no crossover has occurred; that is, the normal and the mutant forms of the locus in question have segregated at the first meiotic division.

Figure 18. Appearance of asci produced upon crossing a wild-type strain of *Neurospora* with a lysine-requiring mutant which exhibits delayed maturation. As discussed in the text, the approximate location of the mutant locus on its chromosome may be deduced from the relative frequencies of first- and second-division segregation. This photograph was obtained through the kindness of Dr. David R. Stadler of the University of Washington.

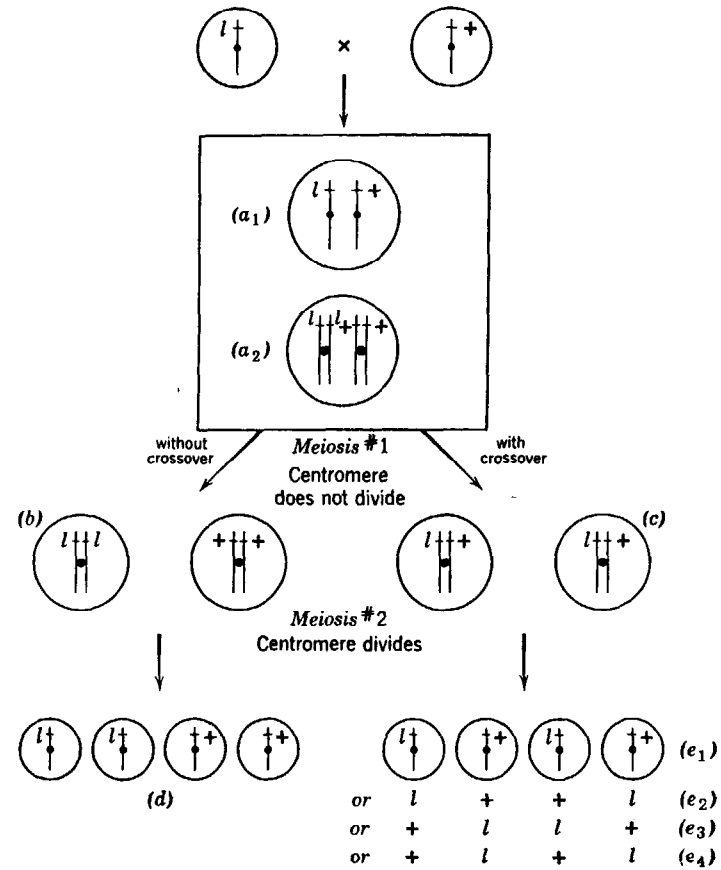

Figure 19. A schematic diagram of the genetic events which occur during the development of an ascus from a zygote in *Neurospora*. The left side of the diagram shows the results of first division segregation, and the right side, those of second division segregation of the two alleles, *l* and +.

Five ascospores show a pattern consistent with *second* division segregation, one alternating as in Figure 19, e_1 and e_4, and four symmetrical as in e_2 and e_3. Therefore, five-fourteenths of the mature ascospores, during development from zygotes, have undergone crossover. Assuming linearity of genes, a direct relationship between crossover frequency and linear distance, and the absence of centromere division in the first meiosis, the mutant locus would be calculated to be $\frac{5}{14} \times 100$ or 36 per cent of the distance from the centromere to the end of the chromosomal strand. (Actually this map distance is to be divided by a factor of two since the unit of mapping in *Neurospora* is defined as one-half of this ratio.)

The crossover frequency values obtained from cross to cross were found by Stadler to vary over a considerable range, as is frequently observed in genetic practice. *Accurate* mapping must always involve a series of crosses between three separate markers or two markers and the centromere, so that additivity may be used as a check. This example is included here because it illustrates how an approximate estimate may be made of the location of a mutant locus in *Neurospora*, even without exhaustive crossing of progeny, when the mutation produces a visible change in the convenient ascospore "recording system."

The great value of the *Neurospora* mutant technique as a tool for relating genetics to biochemistry will be evident from a consideration of the following example. Three genetically distinct mutants, which will grow on the minimal medium when this is supplemented with one or more of the three amino acids, arginine, citrulline, and ornithine, have been isolated. Mutant 1 can grow only when supplied arginine and cannot utilize citrulline or ornithine. Mutant 2 can use both citrulline and arginine, and mutant 3 can manage on any one of the three nutritional additives. These observations suggest that arginine may be produced through the sequence of reactions shown in Figure 20. Assuming the correctness of this biochemical hypothesis, we may propose that the mutant loci in the three mutants each affect a specific enzymatic process in the reaction chain leading to the synthesis of arginine. The correctness of this proposition is indicated by the fact that nutritional mutants will, in general, utilize and grow on intermediates that come after the "block" but not those that precede it. Indeed, in most instances, there is an accumulation of intermediate metabolites preceding the block.

The particular reaction sequence leading to arginine formation is a well-established one for many organisms. The study of the three *Neurospora* mutants is, thus, mainly a confirmatory one, but it has great historical interest since it was one of the earlier clean-cut examples of the direct relation between the enzymatic potential of an organism and its heredity. In many later investigations results derived from the study of other mutants have frequently served as the first wedge in the elucidation of new metabolic pathways.

Perhaps the most significant development growing out of the study of the inheritance of nutritional requirements in *Neurospora* has been the enunciation of the "one gene–one enzyme" hypothesis by G. W. Beadle and E. L. Tatum and their collaborators. This hypothesis, which proposes that a single gene controls the synthesis of only one enzyme or other specific cellular protein, can be made quite flexible by the proper choice of semantics. The breadth of interpretation is

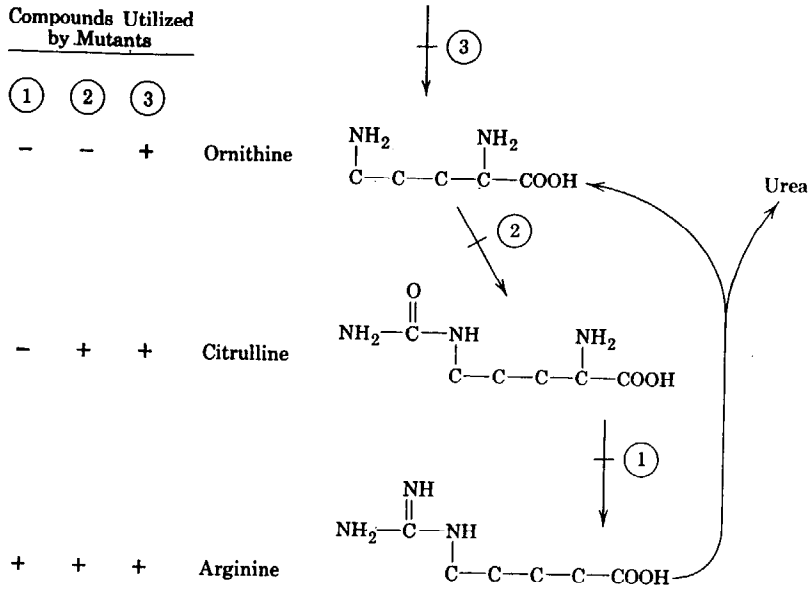

Figure 20. A series of biochemical reactions in the biosynthesis of arginine, the order of which could be established by the study of the nutritional requirements of three mutants of *Neurospora*. From the work of A. M. Srb. and N. H. Horowitz, *J. Biol. Chem.*, **154**, 129 (1944).

directly dependent on the definition we choose to give to the word "gene." Thus, as is true of the pseudoalleles of the "lozenge" gene in *Drosophila*, finer and finer genetic analysis begins to discriminate between loci which are part of the same functional unit. In a relatively coarse analysis, such as the study of the three mutants in the arginine pathway, we are not able to say with certainty whether the blocked step in mutant 2, for example, is immediately prior to citrulline or whether one of a number of intermediate steps between ornithine and citrulline is blocked instead. At the other extreme, an exhaustive genetic analysis might permit the detection of two genetic loci separated by so small a distance along the genetic strand that they would be part of the same functional unit. Mutation of either of these might alter or abolish the biological activity of the same protein molecule. This situation has, indeed, been observed for a number of microorganisms and bacteriophages, and much of what follows in this book will deal with this theme.

One excellent example of a direct relationship between a single protein and a single gene is the case of the two types of tyrosinase in *Neurospora*. Horowitz[7] and his colleagues have shown that the mutation of a single genetic locus causes the formation of a heat-

labile tyrosinase which is indistinguishable from the usual, heat-stable enzyme in all other physical and kinetic properties. The two forms of the enzyme may be isolated in quite pure form, and there can be no doubt that the genetic modification affects a single protein molecule. The difference between the two forms of the enzyme is inherited in a strictly Mendelian way; that is, a given pure strain of *Neurospora* produces only one form of the enzyme, and the progeny of a cross between the two strains are identical with one or the other parent strain in equal proportion.

The possibilities suggested by this and other similar gene-protein relationships are among the most intriguing in the whole of biology. Clearly, if slight modifications in protein structure can ultimately be equated with equally slight changes in the molecular structure of genetic material, there will be opened to us a whole new area of research and speculation on the most basic aspects of the evolutionary process.

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SUGGESTIONS FOR FURTHER READING

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